UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/284,233	07/28/1999	THOMAS F. MEYER	P564-9008	2049

6449 7590 08/01/2003

ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005

EXAMINER	
PORTNER, VIRGINIA ALLEN	
ART UNIT	**PAPER NUMBER**
1645	37

DATE MAILED: 08/01/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/284,233	Meyer et al
Examiner	**Group Art Unit**
Porter	1645

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

Peri d for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

☒ Responsive to communication(s) filed on March 7, 2003.

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 1 1; 453 O.G. 213.

Disp sition of Claims

☐ Claim(s) 24-39 is/are pending in the application.

Of the above claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☐ Claim(s) 24-39 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claim(s) _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Pri rity under 35 U.S.C. § 119 (a)-(d)

☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 11 9(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 1 7.2(a)).

*Certified copies not received: _____.

Attachm nt(s)

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Notice of Reference(s) Cited, PTO-892

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Interview Summary, PTO-413

☐ Notice of Informal Patent Application, PTO-152

☒ Other _____

Office Action Summary

DETAILED ACTION

Claims 1, 3,5-11, 13- 23 have been canceled.

Claims 24-39 have been submitted and are under consideration.

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

CONTINUED EXAMINATION UNDER 37 CFR 1.114 AFTER FINAL REJECTION

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on March 7, 2003 has been entered.

Rejections Withdrawn

3. All previously pending claims have been canceled;

4. All prior rejections, including the Obviousness type Double Patenting rejection, have been withdrawn; newly submitted claims 24-29 are not directed to Helicobacter Alp antigens which were the subject matter rejected under obvious type double patenting.

Response to Arguments

5. Applicant traverses the scope of enablement rejection over claims 1, 3, 5-11, 13-15, 17-22 and 230 on the grounds that "Applicant points out that in the previously submitted declaration the expression of ureaseA and ureaseB was regulated within the plasmid pYZ97 by joint promoters. That is the "cryptic"promoter (cf 2002/0161192 of CIP, paragraph 0065, paragraph 0066, SEQ

ID NO 5, paragraph 0089 and the promoter at position 222-245 in Figure 2) and the T7 promoter."

6. It is the position of the examiner that the instant specification is not the 2002/0161192 of CIP, used to traverse the scope of enablement rejection made of record. The Declarative data presented is based upon embodiments and combinations of reagents not disclosed, suggested or described in the instant specification. Applicant's arguments are not commensurate in scope with the instantly claimed invention which is directed to recombinant attenuated Salmonella cells that comprise a Helicobacter urease nucleic acid; and the recombinant attenuated Salmonella cells are not required to comprise a T7 promoter.

Additionally the Declaration utilized a strain of Salmonella designated SL3261::YZ222. This strain does not evidence original descriptive support in the instant specification. Strain SL3261::YZ222 is described to be a ▲ thyA strain that contains a stabilized plasmid encoding thyA gene as a means for a balanced lethality to complement the chromosomally deleted thyA. This type of mutant strain is not described in the instant specification. Three different promoters for expression were utilized, P_{phoP}, P_{nirB} and P_{T7}. Only P_{T7} evidences original descriptive support in the instant specification, description of P_{phoP}, P_{nirB} could not be found in the instant specification. Four different H.pylori antigens encoded by plasmids in strains CREA 1396, 1398, 1402,1404,1412, 1467, 1468. The strains used to immunize a host were not described in the instant specification. The combinations of expression signals, various antigens and constructs, shown in Exhibit 1 of the Declaration, none of the combinations, were described

in the instant specification. Of the four antigens encoded on the plasmids, only H.pylori urease and heat shock protein were found in the instant specification. Original descriptive support for HylB and citrate synthase homolog was not found for these two antigens.

The strains that evidenced the fewest colony forming units after challenge were CREA 1467 and 1468, which utilized P_{phoP}, or P_{nirB} promoters, both of which do not evidence original descriptive support in the instant specification. The nirB promoter is known to be induced up to 20 fold under anaerobic conditions (see Goldman et al, 1991, reference previously provided).

7. The rejection of claims under 35 U.S.C. 102(b) and (e) as anticipated by Michetti is traversed on the grounds that "Michetti does not indicate that the combination of recombinant urease A and urease B in live vaccines leads to better results that subunits alone.

8. It is the position of the examiner that Michetti discloses the utilization of Helicobacter urease (both A & B subunits) and the subunits of either A or B in the production of recombinant attenuated expression vectors (see col. 19, lines 63-67, col. 20, lines 1-3). There is no need for Michetti et al teach that one is better than the other; Michetti et al discloses the claimed invention directed to a recombinant attenuated Salmonella cell that comprises a heterologous Helicobacter urease (subunits A and B).

9. Applicant asserts that the prior art leads away from the utilization of Helicobacter urease (subunits A and B) together for induction of a protective immune response.

10. It is the position of the examiner that Michetti et al (applied references) taught the utilization of Helicobacter urease (subunits A and B) together for induction of a protective immune response (see col. 19, lines 65-66; col. 22, Table 2, Urease protected 7/10 animals against challenge when urease was administered). Fulginiti et al (reference of record and applied against the claims) taught the utilization of Helicobacter urease for induction of an IgG2a immune response. Monath et al (August 1994, abstract 393) teaches that mucosal immunization with urease resulted in the induction of a protective immune response directed against urease (second half of abstract). The prior art clearly teaches H.pylori urease (urease A and B) induces a protective immune response when administered to a host. Clearly the prior art does not teach away from Applicant's invention.

New Claims/New Grounds of Rejection
Claim Rejections - 35 U.S.C. § 112

11. Claims 24-39 under 35 U.S.C. 112, first paragraph (scope), because the specification, while being enabling for the production and induction of an immune response in an immunocompetent mammal utilizing a recombinant attenuated Salmonella cell that expresses Helicobacter urease A or B or A and B immunogens, does not reasonably provide enablement for the production of recombinant attenuated Salmonella that comprise a heterologous coding sequence for a fragment of Helicobacter urease that is only immunoreactive and is not an immunogen for the induction of a antibody or a protective immune response. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Mimeotopes are conformational epitopes that correspond to a specific receptor or may be represented by linear epitope amino acid sequences, and epitopes by definition are antigens that are from about 3-10 amino acids in size and are immunoreactive with antibodies, but are of a

relative molecular weight less than 1000 daltons and would therefore not be immunogenic. The instantly claimed invention is directed to compositions and methods of making and using the compositions that comprise immunoreactive fragments of Helicobacter urease that include epitopes that are not immunogens for the induction of an immune response, as well as induction of a protective immune response. The instant specification is silent with respect to any specific immunoreactive fragments/epitopes/mimeotopes that would induce a protective immune response.

The term "vaccine" encompasses the ability of the specific antigen to induce protective immunity, in the case of the instantly claimed invention, the protection or prevention of infection would be against pathogenic *Helicobacter*. No specific amounts of antigen are recited in the claims. No specific protective mimeotopes, synthetic peptides that mimic antigen epitopes, are disclosed. A representative number of protective mimeotope species has not been provided to enable the claimed genus of protective mimeotopes for use in vaccine formulations. Data obtained from experiments must demonstrate an art recognized standard of improvement over the control in order for the composition to be considered as being useful for treatment or prevention of infection, especially treatment and prevention of Helicobacter infection. This information is essential for the skilled artisan to be able to use the claimed composition (vaccines) for their intended purpose. Without this demonstration, the skilled artisan would not be able to reasonably predict the outcome of the administration of the claimed vaccines, i.e. would not be able to accurately predict if protective immunity has been induced which would prevent or treat gastric cancer. It is also not clear that any expressed mimeotope/epitope, with a molecular weight of less than 1000 Daltons would be recognized as an immunogen. Mimotopes/epitopes may be as small as 3-5 amino acids and therefore would not evidence a molecular weight high enough to stimulate an immune response, no less a protective immune response, which is necessary to define the composition as a vaccine.

The prior art teaches that Helicobacter pylori vaccines are unpredictable, specifically, in the type of effect they will have on preventing or treating infection; the ability to reasonably

predict the capacity of a single bacterial immunogen to induce protective immunity is problematic. In HP WORLD-WIDE, a publication from Brocades Pharma BV Leiderdorp, The Netherlands, February 1992, data was presented stating that immunization does not appear promising. Parenteral immunization of specific pathogen free mice with *H. felis* gave no protection against gastric colonization; previous oral infection only delayed colonization (Heap,K, Australia). The article also taught that "although intra-peyers patch immunization of killed *H. pylori* in rats shows that the gut mucosa can mount a vigorous immune response, oral immunization with either live or killed bacteria induced no significant serum or salival antibody response (Dunkley, M, Australia). Blaser also warned that because of the possible autoimmune component of the disease the wrong vaccine could actually make things worse." Accordingly, the art indicates that it would require undue experimentation to formulate and use a successful vaccine without the prior demonstration of vaccine efficacy.

Given the lack of guidance on how to obtain the desired effect using any composition comprising any active ingredient for the prevention or therapeutic vaccine in a method of treating any disease caused by Helicobacter in light of the teachings of the prior art which teaches that vaccines comprising Helicobacter antigens are unpredictable in methods of treating or preventing infection the skilled artisan could not make and use the claimed invention. No evidence is of record showing that **any** composition could confer the desired and claimed effect. No working examples are shown which convey the missing information or show the nucleic acid sequences that upon expression as a heterologous antigen in an attenuated bacterium would serve to induce a protective immune response against Helicobacter infection and disease. Therefore, the skilled artisan could not use **any** recombinant bacterium that comprises a heterologous nucleic acid that would express the encoded Helicobacter antigen, or mimotope of said antigen, to obtain the desired effect of preventing or treating infection without undue experimentation.

12. Claims 24,26-29, 31 and 33 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 24 and 31 recite the phase "immunologically reactive fragments". This phrase lacks antecedent basis in claims 24 and 31, respectively, which recite "Helicobacter immunogen". How can an immunologically reactive fragment define a protective immunogen if it is not immunogenic, and is only immunoreactive? Epitopes are immunoreactive fragments that are not immunogenic. The "wherein" statement which recites limitations directed to immunologically reactive fragments broadens the scope of claims 24 and 31 to include epitopes that are not immunogens. The invention is not distinctly claimed.

Claim 26 broadens the scope of claim 24 through the recitation of the phrase "a Helicobacter immunogen"; the term "a" is a non-specific article and does not refer back specifically to --the-- Helicobacter urease of claim 24. This rejection could be obviated by amending the claim to recite --said Helicobacter urease immunogen--, or another phrase that refers specifically to the Helicobacter urease immunogen of claim 24.

Claim 26 recites the phrase "is phase variably expressed" and depends from claim 24 which does not comprise any components that are operatively linked to the heterologous nucleic acid that would result in the Helicobacter immunogen being variably expressed. The invention is not distinctly claimed. Clarification of the invention is requested.

Claim 27 should recite the phrase --further comprises-- an additional heterologous nucleic acid that is a control expression signal. Claim 24 does not define the presence of a control signal. Claim 27 must therefore --further comprise -- the nucleic acid for "nucleic acid reorganization". The control expression signal only activates the expression of the immunogen recited in claim 24, what controls the expression of the "fragments" of claim 24 that are not immunogens?

Claim 28 should recite the phrase --further comprises a nucleic acid encoding a bacteriophage RNA polymerase--, as the host cells of claims 24 and 27 do not comprise the essential structural components that are required for the recited functional characteristics.

Claim 29 recites the phrase " further comprises at least one second nucleic acid molecule". As Salmonella comprises a plurality of nucleic acid molecules on its bacterial chromosome, and is capable of expressing at least the second nucleic acid molecule to remain viable, how is claim 29 further limiting of claim 24 which is directed to a Salmonella cell that comprises a plurality of nucleic acid molecules?

Claim 33 recites the phrase "a recombinant attenuated cell according to claim 24"; the term "a" is a non-specific article and therefore does not distinctly claim Applicant's invention. An attenuated Salmonella is a species of recombinant attenuated cell and would meet the recited claim limitations of claim 33. The claimed living vaccine need not comprise the heterologous Helicobacter coding sequence for urease based upon the limitations set forth in claim 33. Claim 33 reads on an attenuated Salmonella, thus broadening the scope of claim 24 from which it depends. This rejection could be obviated by amending the claim to recite --the-- prior to the word "recombinant".

Claim Rejections - 35 U.S.C. § 102

Case Law applicable to rejections set forth below:

Since the Office does not have the facilities for examining and comparing applicant's protein with the protein of the prior art, the burden is on applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the protein of the prior art does not possess the same functional characteristics of the claimed protein). See In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and In re Fitzgerald et al., 205 USPQ 594

Inherently the reference anticipates the now claimed invention. Atlas Powder Co. V IRECA, 51 USPQ2d 1943, (FED Cir. 1999) states "Artisans of ordinary skill may not recognize the inherent characteristics or functioning of the prior art...However, the discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior art's functioning, does not render the old composition patentably new to the discoverer. "The Court further held that "this same reasoning holds true when it is not a property but an ingredient which is inherently contained in the prior art".

13. Claims 24-26, 29-39 are rejected under 35 U.S.C. 102(b) as being anticipated by Fulginiti et al (September 13-17, 1995).

(Instant claims 24-26, 29, 33-35) Fulginiti et al disclose the claimed invention directed to an attenuated AroA Salmonella cell (abstract (ab).line 4) expressing a heterologous Helicobacter pylori urease (ab.line 1) protein (ab.line 4). The heterologous Helicobacter pylori urease coding sequence (urease A and B subunits) was inserted into a plasmid (ab.line 8) (pPX5024) and operatively linked to a plasmid sequence for expression (ab.line 8). The expression of the Helicobacter pylori immunogen was carried out during growth phase (verses stationary phase).

(Instant claims 34 and 35) The Salmonella compositions were formulated for mucosal (intragastrically; ab.line 7) and intraperitoneal administration (see ab.lines 11-12). and 10^{10} cells were administered mucosally and 10^{6} AroA mutant recombinant Salmonella SL3261/pPX5024 were administered parenterally to a mammal and stimulated

Recombinant Salmonella cells were recovered from multiple locations in the patient/mice,(ab. line 7, and 19), Recombinant cells were recovered from peyer's patches, livers and spleens of the patient (mice; ab.line 19). Additionally the host cell comprised at least a second nucleic acid molecule encoding an immunomodulatory polypeptide, specifically Salmonella immunogenic proteins and antigens that are surface expressed.

Both mucosal and serum immune responses (IgA and IgG;ab.lines 16 and 29) specific to the Helicobacter urease immunogen were detected in the patient/mammal.
(Instant claim 30) A method was carried out in order to formulate the living vaccine composition for administration to a patient, the method comprising the steps of:

providing a recombinant attenuated AroA Salmonella strain that comprises a Helicobacter nucleic acid encoding a urease immunogen (ab.line 4); and

formulating the living cell that comprises a carrier (cell membrane/cell wall;ab.line 2) into a composition for induction of a protective immune response (ab.line29).
(Instant claim 31-32) The recombinant attenuated AroA Salmonella strain that comprises a Helicobacter nucleic acid encoding a urease immunogen was prepared by a method comprising the steps of:

inserting a nucleic acid encoding a Helicobacter urease (Helicobacter urease has A and B subunits) into an attenuated AroA Salmonella cell (ab.line"transformed",line8); the Helicobacter coding sequence being located on an extrachromosomal plasmid (see ab.line 8) and

culturing the recombinant attenuated AroA Salmonella strain under suitable conditions (10^{10} were produced by culturing for administration;ab.line 7).
(Instant claims 36-39) A method for treating (encompasses prevention/ therapeutic for pre-existing infection/inducing a protective immune response) an infection by Helicobacter pylori infection, the method comprising the step of:

administering a composition to a patient (ab.line 6) a recombinant attenuated AroA Salmonella strain for inducing protective immunity against Helicobacter pylori, wherein the composition is administered as a single dose (10^{10} cells were administered as a single dose, see ab.line 7). The composition administered to a mammal, was in an amount effective to induce both an IgA and IgG immune responses. The living vaccine composition comprised a carrier,

specifically the cell membrane, which served to carry the Helicobacter pylori coding sequence plasmid. The immunity induced was primarily IgG2a (ab.line 29).

Fuliginiti et al anticipates the instantly claimed invention.

14. Claims 24, 26, 29, 33-35 are rejected under 35 U.S.C. 102(e) as being anticipated by Michetti et al (US Pat. 6,290,962, filing date Feb. 1994).

(Instant claims 24, 26, 29, 33-35) Michetti et al (US Pat. 6,290,962) disclose the instantly claimed invention directed to a recombinant attenuated (see col. 9, lines 24-25) Salmonella (see claims 52 and 67, as well as col. 9, lines 23-24) composition that comprises a heterologous Helicobacter nucleic acid molecule encoding a Helicobacter urease immunogen (See Michetti claims 41-70), together with a second immunomodulatory immunogen (see col. 9, line 20, urease fusion with cholera toxin) , wherein the composition is formulated together with a second nucleic acid molecule that is an immunomodulatory polypeptide (see Michetti, claims 45-46,51, 60-61), or formulated together with a diluent, carrier or adjuvant (see Michetti claims 46-50, 60-66) and would be suitable for mucosal or parenteral administration (see col. 9, lines 35-37; includes saline can be used for mucosal or parenteral administration).

The Helicobacter pylori heterologous nucleic acid is "incorporated into the genetic material of the live vector (see col. 18, lines 33-34) or inserted into a plasmid vector (see col. 19, lines 19-30) followed by the insertion of the plasmid into a prokaryotic expression vector (see col. 20, line 2), which includes the disclosed attenuated Salmonella cell.

15. Claims 30-32 and 36-39 are rejected under 35 U.S.C. 102(e) as being anticipated by Michetti et al (US Pat. 6,290,962, filing date Feb. 1994).

(Instant claim 30) The attenuated Salmonella cell is used in a method of preparing a vaccine, the method comprising the steps of:

providing the attenuated Salmonella cell that comprises a nucleic acid encoding a Helicobacter urease (see col. 9, lines 23-25 and see claims 52 and 67); and

formulating the cell into a composition together with a diluent, carrier or adjuvant (see col. 11, lines 46-52; col. 12, lines 16-32; col. 13, lines 25; col. 13, lines 47-67 and col. 14, lines 1-14; col. 19, lines 63-67, col. 20, lines 1-3, 13; see col. 8, lines 63-65 and col. 9, lines 35-65). (Instant claims 31-32) Michetti et al disclose the claimed invention directed to methods of preparing a recombinant attenuated Salmonella, the methods comprising the steps of:

inserting a nucleic acid encoding a Helicobacter urease into an attenuated Salmonella cell, wherein the nucleic acid is in a plasmid (see col. 19, line 20) or inserted in the chromosome (see col. 18, lines 32-33); and

cultivating the recombinant attenuated Salmonella cell (see col. 19, lines 19-67 and col. 20, lines 1-3).

(Instant claims 36-39) Michetti et al (US Pat. 6,290,962) disclose methods of using an attenuated pathogen for inducing a protective immune response, that results in treating or preventing infection the methods comprise the step of:

administering the composition that comprises an attenuated Salmonella composition that encodes a Helicobacter immunogen. (see col. 12, lines 16-32; col. 13, lines 56-67 and col. 14, lines 1-14; col. 18, lines 28-49; claims 1-40 and claims 71-72; col. 14, lines 15-67).

16. Claims 24, 26, 33-35 are rejected under 35 U.S.C. 102(b) as being anticipated by Michetti (WO95/22987).

Michetti et al disclose the instantly claimed invention directed to a recombinant attenuated Salmonella cells (genetically engineered attenuated live vectors, see page 23, lines 11-12), specifically Salmonella typhimurium, Salmonella typhi (see pages 35-36, line bridging these pages). The compositions would comprise a carrier liquid such as saline or sodium bi-carbonate(page 23, lines 22-25) and enteric coated capsules or microspheres. The formulation

of the compositions defines means for mucosal or parenteral administration, specifically aerosol, suspension, capsule or suppository (page 25, lines 5-15). Attenuated Salmonella cells are disclose to comprise heterologous Helicobacter pylori urease subunits (see page 53, lines 5-6). Inherently the reference discloses the now claimed invention (see Michetti, claims 25-26 and 61-62).

Claim Rejections - 35 U.S.C. § 103

17. Claims 24-29, 33-35 are under 35 U.S.C. 103(a) as being unpatentable over Michetti (WO95/22987) in view of Russell et al (US Pat. 6,030,624).

See discussion of Michetti above. Michetti et al teach the production and formulation of attenuated Salmonella cells that comprise an heterologous Helicobacter urease coding sequence that is phase variable expressed (growth phase vs stationary phase; and/or in association with a thermo-repressible promoter (pEV40 plasmid comprised this type of promoter), but differs from the instantly claimed invention by failing to show the utilization of a T-7 bacteriophage promoter.

Russell et al show the formulation of attenuated live S.typhimurium delta-AroA, delta-AroD mutant vectors under the control of bacteriophage T7 transcription (col. 3, lines 55-63 and claims) for the expression of heterologous DNA from Helicobacter pylori (col. 9, lines 28-30, lines 35-37, lines 39-45 and lines 66) linked to Vibrio cholera toxin A2/B subunits nucleic acid sequences in an analogous art for the purpose of recombinantly producing and expressing chimeric proteins for stimulating a mucosal immune response (abstract, figures, col. 3, lines 37-46; col. 1, lines 45-67 and col. 2, lines 1-3) against pathogens, to include Helicobacter and Salmonella.

Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify recombinant Salmonella of Michetti with the T-7 promoter of Russell et al because Russell et al teach that through the utilization of a T-7 promoter the attenuated AroA live Salmonella host cell vectors are able to induce a mucosal

immune expressed heterologous DNA, provide means for antigen presentation directly to mucosal inductive surfaces without the need for antigen purification (col. 3, lines 50-55) prior to presentation to a patient/mammal in a method of inducing an immune response.

The person of ordinary skill in the art would have been motivated by the reasonable expectation of success of obtaining a recombinant attenuated Salmonella that comprises a Helicobacter urease and a second immunomodulatory polypeptide under the control of a bacteriophage promoter expression signal because Russell et al clearly teaches the advantage of utilizing non-toxic chimeric proteins which comprise CTA2/B fusion proteins for the induction of IgA antibodies to protect mucosal surfaces of the gastrointestinal tract, and provides guidance for the use of attenuated recombinant host cells which express a heterologous antigen in order to overcome the problems associated with oral immunization of protein immunogens which become denatured by gastric acid and digestive enzymes, have limited adsorption by the intestinal mucosa and clearance by peristalsis. A recombinant attenuated Salmonella host cell which is avirulent would function as a live vaccine delivery system with tropism for the gut associated lymphoid tissue and Helicobacter is a known mucosal pathogen which induces chronic infection leading to gastritis and gastric ulcers.

The combination of Michetti in view of Russell et al teach recombinant attenuated Salmonella host cells for the expression of Helicobacter antigen linked to a second heterologous nucleic acid that encodes an immunogenic cholera toxin A2/B subunit. Michetti clearly teaches the use of attenuated strains of bacteria for the expression of heterologous Helicobacter DNA and claims the use of recombinant Salmonella and Russell provides motivation to the person of ordinary skill in the art by teaching means, methods and modes of administration of expecting reasonable success in stimulating an enhanced mucosal immune response against Helicobacter due to the presence of a second immunomodulatory polypeptide.

In the absence of a showing of unexpected results, the person of ordinary skill in the art would have been motivated by the reasonable expectation of success of obtaining a

chimeric recombinant Helicobacter antigen-A2/B cholera toxin fusion protein because Russell suggests the production of a Helicobacter antigen/A2-B cholera chimeric protein and recombinant DNA, as well as teaches means, methods, expression vectors, host cells and multiple advantages of utilizing a chimeric protein and a recombinant attenuated host cell which expresses the chimeric protein for inducing a mucosal immune response which would be provide for reducing or preventing infection caused by Helicobacter.

Conclusion

18. This is a non-final action.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ginny Portner whose telephone number is (703)308-7543. The examiner can normally be reached on Monday through Friday from 7:30 AM to 5:00 PM except for the first Friday of each two week period.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909. The fax phone number for this group is (703) 308-4242. The Group and/or Art Unit location of your application in the PTO will be Group Art Unit 1645. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to this Art Unit.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Vgp

July 22, 2003

NITA MINSFIELD
PRIMARY EXAMINER
7/24/03

391

VALIDATION OF A MODIFIED KIRBY-BAUER DISK DIFFUSION METHOD FOR METRONIDAZOLE SUSCEPTIBILITY TESTING OF HELICOBACTER PYLORI.

P.D.Midolo[1*], J.Turnidge[1], J.R.Lambert[2]

Department of Microbiology and Infectious Diseases[1], Monash Medical Centre, Clayton and Gastroenterology Research Group[2], Mornington Peninsula Hospital, Frankston, Australia.

Triple therapy including metronidazole has been recommended as a first-line therapy with good eradication rates of H.pylori. Resistance in H.pylori to metronidazole has been reported worldwide. Various methods for testing H.pylori against metronidazole have been used including agar dilution, disk diffusion and the E-test but there has been little standardization of methods.

Methods: One hundred and six isolates of H.pylori from consecutive patients were tested for susceptibility to metronidazole by agar dilution (following NCCLS guidelines), E-test and disk diffusion (5ug disk). All three methods used Wilkens-Charlgren agar with 5% horse blood and were performed simultaneously from a 1 McFarland suspension of organisms in BHI broth.

Results/Conclusions:The agar dilution results confirmed the MIC susceptibility breakpoint to be <8mg/l. Using this breakpoint there was close agreement (93%) between E-test and agar dilution results. For susceptible strains, MICs by E-test were generally one twofold dilution lower. Agreement between disk diffusion zone diameter and MIC was 93% for agar dilution with breakpoints of >13mm and <8mg/l and 98% for E-test with breakpoints of >10mm and <8mg/l. The E-test discriminated better than agar dilution between susceptible and resistant strains and was simple to perform. The disk diffusion test is a reliable and cheap alternative to the E-test with susceptibility being a zone [illegible]. The prevalence of metronidazole [illegible].

392

PHOTODYNAMIC THERAPY FOR THE TREATMENT OF HELICOBACTER IN THE FERRET STOMACH

C.E.Millson, M.Wilson, A.J.MacRobert and S.G.Bown.
National Medical Laser Centre and the Institute of Dental Surgery, University of London.

Antibiotic treatment for *H. pylori* is not entirely satisfactory. As we have already demonstrated that *H. pylori* can be killed by lethal photosensitisation in vitro, the purpose of this study was to determine the efficacy of this therapy, using the ferret model.

Explanted ferret stomach tied at the duodenum was filled with 2 mls of sensitiser, of varying concentrations, [Haematoporphyrin derivative, Phthalocyanine, Methylene blue (MB) or Toluidine blue] and the cardia tied. One hour later, the stomach was opened and the antrum divided into 4 strips 1 x 0.5cm. Each strip was halved, and one square exposed to light from the copper vapour laser at varying energy doses, whilst the other square was used as a control. All strips were placed in saline, homogenised and serially diluted (Miles and Misra) to obtain a viable count. One ferret was given oral Aminolaevulinic acid (ALA, 750mg/kg) 6 hours prior to sacrifice and treated as described above, to observe the effect of the endogenous sensitiser protoporphyrin IX (PPIX). A control stomach was processed to observe the effect of the laser light alone or no intervention.

MB at 50μg/ml combined with 50J/cm^2 laser light resulted in a 99% reduction in viable count, whilst increasing the MB concentration to 5000μg/ml resulted in complete eradication at the same energy dose. ALA treatment resulted in a 95% kill (160J/cm^2 energy dose) but none of the other sensitisers achieved significant kill. MB did exhibit some dark toxicity upon *Helicobacter mustelae* at 5000μg/ml but laser alone had no effect.

Exposure to low-power laser light kills *H. mustelae* sensitised by MB and PPIX, in the ferret stomach and raises the possibility of an alternative, non-antibiotic, method of eradication if it proves possible to treat all infected areas in vivo.

393

PROGRESS TOWARDS A VACCINE AGAINST *HELICOBACTER PYLORI*

TP Monath, W Thomas, RA Weltzin, G Soman, SA Ackerman, J Pappo, T Ermak, H Bhagat and CK Lee, OraVax, Inc., Cambridge MA.

Helicobacter pylori is one of the most prevalent infections of humankind and an important cause of gastrointestinal diseases worldwide. Because it is a chronic infection that persists lifelong while eliciting strong immune responses to multiple antigens, the feasibility of vaccination (particularly post-exposure vaccination) has been questioned. However, Czinn and Nedrud (*Vaccine* 1993; 11:637) and Chen et al. (*Lancet* 1992; i:1120) demonstrated that oral immunization with lysates of *H. felis* protected mice against hololgous challenge, and Michetti et al. have demonstrated that recombinant *H. pylori* ureB complexed to hydroxylapatite is similarly effective (*Gastroenterology*, in press). We developed an efficient system for the expression of *H. pylori* urease apoenzyme in *E. coli*, and methods for its purification and stabilization as a mucosal vaccine. The recombinant apoenzyme was shown to retain both the ultrastructural integrity of native holoenzyme and reactivity with a protective monoclonal antibody. When administered into the oral cavity of outbred mice, 4 doses of 5 μg given at intervals of 1 week provided highly significant protection against subsequent challenge with *H. felis*, and doses of 25 μg were 100% protective. An adjuvant was required for protection, however, and this requirement could not be eliminated by administering high doses of antigen; cholera toxin and labile toxin of *E. coli* were effective adjuvants, but a derivative of muramyl dipeptide was not. Parenteral administration of urease induced a strong serum IgG response that was not protective. In contrast, immunization by mucosal routes elicited anti-urease serum, fecal and salivary IgA antibody responses that correlated with protection. The results indicate that prophylactic oral vaccination is feasible, that a subunit antigen (urease) is effective, and that secretory IgA mediates protection. Other studies will be reported on the precise role of cellular and humoral immunity in protection; on the identification of protective [illegible], on persistent infection after immunization; on the vaccination of animal models such as *H. pylori*; and on strategies for eliminating the requirement for adjuvants.

394

ABSENCE OF EFFECT OF ERADICATION OF *HELICOBACTER PYLORI* ON GASTRIC ULCER RELAPSE, UNLIKELY TO DUODENAL ULCER.

Hiroko Nebiki, Tetsuo Arakawa*, Hideaki Yamada, Kiyotaka Ohkawa, Shigeyoshi Harihara, Hiroyuki Ito*, Kazuhide Higuchi*, Kenzo Kobayashi*, Gastroenterology Osaka City General Hospital and The third department of Internal Medicine Osaka City University Medical School*, Osaka, Japan

Purpose; Relapse rate of duodenal ulcer is markedly small after eradication of *Helicobacter pylori* (*Hp*). However, effect of the eradication on gastric ulcer relapse is still not clear. Here we examined whether the eradication of *Hp* is effective for prevention of gastric ulcer relapse or not in Japan. **Patients and methods;** The 28 patients with gastric ulcer and 11 patients with duodenal ulcers infected with *Hp* were assessed. Presence of *Hp* was evaluated by histology, culture, and CLO test. They were treated first with omeprazole 20 mg together with amoxicilin 1500 mg for two weeks and then, with omeprazole alone for 6 weeks. Endoscopy was performed before treatment, during treatment (at 4 and 8 weeks), and after treatment (every 2 or 3 months) to examine ulcer healing and relapse and presence of *Hp*. **Results;** Healing rate of gastric ulcer was 93% and that of duodenal ulcer was 100%. The eradication rate was 42.9% (12/28) in gastric ulcer patients and 45.5%(5/11) in duodenal ulcer patients. In gastric ulcer, cumulative remission rate was the same between the groups with successful eradication of *Hp* and those without (Fig. 1), while the rate was makedly higher when the eradication was succeeded in patients with duodenal ulcer (Fig. 2).

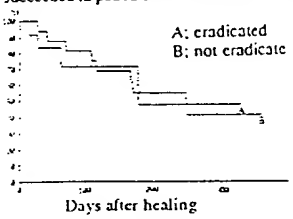


Fig.1 Cumurative remission rate of gastric ulcers.

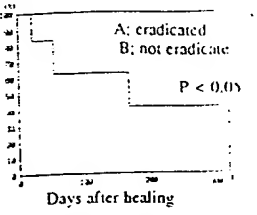


Fig.2 Cumurative remission rate of duodenal ulcers.

Conclusions; *Hp* may not have a crucial role in gastric ulcer relapse, unlikely to duodenal ulcer, at least in Japan.

INTERLIBRARY LOAN REQUEST FORM

Borrower's Name: Portner | Org. or A.U.: 1641 | 30[illegible]3

Serial Number: PCT/US98/ Hpylo | Date of Request: 6/3/1[illegible] | Date Needed By: [illegible]

Please Attach Copy of Abstract, Citation, Or Bibliography. If Available Please Provide Complete Citation. Only One Request Per Form.

Author/Editor:	Monath T [illegible]
Journal/Book Title:	Am J [illegible]
Article Title:	A[illegible]
Volume (Issue):	89
Pages:	1380 - 13[illegible]
Year of Publication:	1994
Publisher:	
Remarks:	

STIC Use Only

Accession Number: 816380

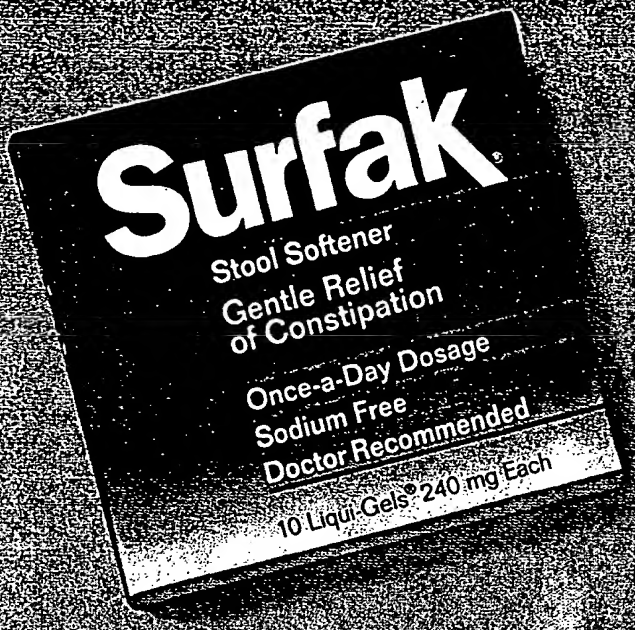
LIBRARY ACTION	LC		NAL		NIH		NLM		NBS		PTO		OTHER	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Local Attempts	✓													
Date					6/5						6/4			
Initials					SMP						AD			
Results					Completed									
Examnr. Called					0									
Page Count					2									
Money Spent					20									

Provided By: Source and Date

Ordered From: Source and Date

Remarks/Comments
-1st & 2nd denotes times taken to a library
-FX - Means Faxed to us
-O/N - Under NLM=Overnight Service

The UnColace.



SURFAK is priced less and it's once-a-day.

If you take everything you like about Colace–but lower the cost, make the dosage more convenient, and take out the sodium–you have a better stool softener to recommend to your patients.

You have SURFAK.

	SURFAK®	COLACE®
COST PER DAY	27.2¢	68.8¢
CAPSULES PER DAY	1	2
MG SODIUM PER DOSE	0	10.4
CLINICALLY EFFECTIVE INGREDIENT	Docusate Calcium	Docusate Sodium

SURFAK offers safe, effective prevention and relief of hard stools for your surgical and postpartum patients who need gentle laxation.

Plus, SURFAK is less than half the cost of Colace, has a convenient dose of one capsule a day, and is sodium free.

Recommend it for anyone who needs the reliable action of a proven stool softener.

Surfak®

Stool Softener

The cost-effective alternative to Colace.